Regional Cortical Thinning Associated with Detectable Levels of HIV DNA

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High levels of human immunodeficiency virus (HIV) DNA in peripheral blood mononuclear cells (PBMCs), and specifically within CD14+ blood monocytes, have been found in HIV-infected individuals with neurocognitive impairment and dementia. The failure of highly active antiretroviral therapy (HAART) to eliminate cognitive dysfunction in HIV may be secondary to persistence of HIV-infected PBMCs which cross the blood-brain barrier, leading to perivascular inflammation and neuronal injury. This study assessed brain cortical thickness relative to HIV DNA levels and identified, we believe for the first time, a neuroimaging correlate of detectable PBMC HIV DNA in subjects with undetectable HIV RNA. Cortical thickness was compared between age- and education-matched groups of older (>40 years) HIV-seropositive subjects on HAART who had detectable (N = 9) and undetectable (N = 10) PBMC HIV DNA. Statistical testing revealed highly significant (P < 0.001) cortical thinning associated with detectable HIV DNA. The largest regions affected were in bilateral insula, orbitofrontal and temporal cortices, right superior frontal cortex, and right caudal anterior cingulate. Cortical thinning correlated significantly with a measure of psychomotor speed. The areas of reduced cortical thickness are key nodes in cognitive and emotional processing networks and may be etiologically important in HIV-related neurological deficits.

Keywords: cortical thickness, insula, magnetic resonance imaging, neurocognitive impairment, PBMC

Introduction

In developed countries, highly active antiretroviral therapy (HAART) has transformed human immunodeficiency virus (HIV)/AIDS from a subacute lethal disease to a chronic illness (Hammer et al. 1997; Palella et al. 1998) by dramatically reducing opportunistic infections and AIDS-related mortality (Moore and Chaixson 1999). Initiation of HAART suppresses plasma HIV RNA (viral load) and restores immune function (Brodt et al. 1997). However, despite decreased incidence of HIV-associated dementia (Brodt et al. 1997; Sacktor et al. 2001), milder neurocognitive deficits related to the infection remain a major concern in this population. Prevalence of cognitive dysfunction in patients on HAART is estimated at 20–37% (Sacktor et al. 2001, 2002; Robertson et al. 2007). Individuals on HAART, whose plasma viral loads are low or undetectable, are nevertheless susceptible to HIV-induced neuronal damage (Neuenburg et al. 2002) and may suffer impaired cognition (Cysique et al. 2004a, 2004b). Although neurocognitive function can markedly improve with HAART (Sacktor et al. 2000; Suarez et al. 2001; McArthur et al. 2003; Robertson et al. 2004), deficits typically fluctuate in severity (McArthur et al. 2003; Antinori et al. 2007). One longitudinal study showed that HIV-infected (HIV+) subjects failed to return to premorbid functioning after 3 years of treatment (Anderson et al. 2002). HIV-associated neurocognitive impairment diminishes quality of life even in the era of HAART (Tozzi et al. 2003, 2004).

Incomplete cognitive recovery has heightened the need to understand mechanisms of HIV-associated neurocognitive disorders (HANDs). Neurological damage results primarily from neurotoxins released by macrophages and microglia (Heyes et al. 2001; Kaul et al. 2001; Li et al. 2005; Mattson et al. 2005) rather than from direct HIV infection. Plasma HIV RNA (viral load) is typically well controlled by HAART. However, even with suppression of plasma viremia, HIV-infected cells (including HIV-infected monocytes) continue to be found in the bloodstream of many individuals. Cell-free plasma HIV RNA and HIV-infected cells (HIV DNA) appear to play biologically different and independent roles in HIV pathogenesis (Scott-Algar et al. 2010). HIV-infected monocytes are of particular interest because they have been implicated in the pathogenesis of HAND (Gonzalez-Scarano and Martin-Garcia 2005). Monocytes are believed to become activated upon exposure to HIV or its components. Activated monocytes, including those that are infected, cross the blood-brain barrier to accumulate in perivascular spaces where they initiate microglial activation and inflammatory processes (Liu et al. 2000; Kim et al. 2003). Activated monocytes that transmigrate the blood-brain barrier are therefore integral to the pathogenesis of HAND (Koenig et al. 1986; Gartner 2000).

HIV-infected cells in the bloodstream were quantitated by assessing the amount of HIV DNA present per 10^6 peripheral blood mononuclear cells (PBMCs) (Shiramizu et al. 2005). HAART-treated patients whose plasma viral loads are undetectable by current assays can have detectable PBMC HIV DNA levels (Wong et al. 1997). When therapy is initiated, plasma HIV RNA decline precedes and is steeper than the drop in PBMC HIV DNA (Bruisten et al. 1998). HAART reduces HIV DNA more effectively when initiated early in the course of infection (Ngo-Giang-Huong et al. 2001; Hocquoloux et al. 2009). PBMC HIV DNA load may indicate the spread of disease, whereas plasma HIV RNA reflects active infection (Kostrikis et al. 2002; Re et al. 2006).

HIV DNA in PBMCs constitutes a viral reservoir that contributes to ongoing neurological impairment. Detectable PBMC HIV DNA correlates with cognitive dysfunction both in HAART-naïve individuals (Shiramizu et al. 2007a) and in HAART-treated subjects with undetectable plasma HIV RNA (Valcour et al. 2009, 2010). Recent data suggest that HIV DNA...
is an independent risk factor for HIV-associated neurocognitive impairment (Carsenti-Dellamonica et al. 2011). Activated monocytes that cross the blood-brain barrier are involved in the pathogenesis of HAND (Koenig et al. 1986; Gartner 2000). HIV DNA likely reflects the presence of HIV DNA within monocytes (CD14+ cells). Cognitive decline was correlated with HIV DNA specifically within the activated CD14+ monocyte subset of PBMCs (Shiramizu et al. 2007a; Valcour et al. 2009), indicating that these migrate to the brain and set up an environmental milieu that is primed for neuronal injury.

Studies using brain volumetric magnetic resonance imaging (MRI) show that major targets for HIV include subcortical gray matter structures (e.g., basal ganglia, thalamus) and central white matter (Aylward et al. 1993; Hall et al. 1996; Stout et al. 1998). Concomitant widespread cortical atrophy is found in HIV-infected subjects compared with healthy controls (Thompson et al. 2005; Chiang et al. 2007). Primary sensorimotor cortices are thinned by about 15%, and prefrontal and parietal gray matter atrophy is linked with cognitive and motor impairment (Thompson et al. 2005). Cortical as well as subcortical atrophy persists despite effective antiretroviral treatment (Cohen, Harezlak, Schifitto, et al. 2010; Becker et al. 2011). HIV-related brain volumetric loss in HAART-treated patients with suppressed plasma viral load may present a somewhat cortical pattern; and whereas basal ganglia shrinkage seems most related to current disease status, cortical and global brain volumes correlate most strongly with disease history variables (Cohen, Harezlak, Schifitto, et al. 2010). Cortical involvement (reduced resting blood flow in the visual cortex) was observed via arterial spin labeling perfusion MRI in HIV+ individuals (Ances et al. 2009). Proton magnetic resonance spectroscopy (MRS) reveals HIV-associated cerebral metabolite disturbances; for example, decreased levels of N-acetylaspartate (NAA) that indicate neuronal injury and increases in choline and myo-inositol reflecting inflammation (Chang, Lee, et al. 2004; Schweinsburg et al. 2005; Paul et al. 2007; Lentz et al. 2009; Schifitto et al. 2009; Mohamed et al. 2010; Harezlak et al. 2011). Such metabolite abnormalities, especially loss of NAA, were reported to be significantly associated with both cortical and subcortical atrophy in HIV, though the relationship between biochemical and volumetric changes is not well understood (Cohen, Harezlak, Gongvatana, et al. 2010).

While monocyte reservoirs contribute to the continued prevalence of cognitive dysfunction in HIV infection, the relevance to HAND of lymphocytes, which also migrate to the brain, is unclear (Kalams and Walker 1995; Kaul et al. 2005). In the current work, we focus on HIV DNA in PBMCs. Our MRI-based study examined cortical thickness in HIV+ subjects who had detectable HIV DNA levels, using as a comparison group HIV− individuals whose HIV DNA was undetectable. All participants were on HAART with evidence of viral suppression. We present here what we believe is the first report in the literature of a neuroimaging correlate of detectable HIV DNA in peripheral blood.

Materials and Methods

Subjects

This retrospective study was based on a convenience sample of MRIs from HIV-infected individuals in a cross-sectional study that explored the relationship of brain metabolites to PBMC HIV DNA using MRS. (MRS-derived findings will be reported in a separate publication.) Participants with undetectable levels of PBMC HIV DNA (<10 copies/10⁶ cells) and detectable HIV DNA (>10 copies/10⁶ cells) were enrolled. Each subject provided written informed consent for data and specimens to be utilized for other studies related to HIV and cognitive dysfunction. The MRS study and ancillary consent for future use of data and specimens were approved by the University of Hawaii Committee on Human Studies. All subjects had documented evidence of HIV infection. Exclusion criteria for the 1H MRS study included any major psychiatric or neurological disorder, history of head injury with unconsciousness lasting longer than 30 min, learning disability, current substance abuse or dependence as defined by the Diagnostic and Statistical Manual of Mental Disorders, fourth edition (DSM-IV) (American Psychiatric Association 1994), history of opportunistic brain infection, primary language other than English, and implanted metal or other conditions (e.g., claustrophobia) precluding the use of MRI. Subjects underwent neuroimaging, clinical evaluations, blood draws for assays of PBMC HIV DNA, and neuropsychological testing from September through October 2008. T₁-weighted MRS was performed as part of the protocol. Specimens were obtained and stored at the time of study entry. Plasma HIV RNA and CD4 cell counts were performed by a local commercial CLIA-certified laboratory. Nadir CD4 count and years since HIV diagnosis were determined by subject self-report.

PBMC HIV DNA Assessment

Blood draws for PBMC HIV DNA copy assays were performed within 30 days of MRI. The HIV DNA assay was performed as previously reported (Shiramizu et al. 2005). Its low intra-assay and inter-assay variability is indicated by mean coefficients of variation of 1.1% and 1.4%, respectively. Copy numbers of each sample gene (HIV gag and beta-globin) were analyzed against the standard curves, and the HIV DNA copy number per 1 × 10⁶ cells determined. The lower limit of detection of HIV DNA was 10 copies/10⁶ cells, with values less than 10 copies/10⁶ cells considered undetectable.

Neuropsychological Assessment

Neuropsychological testing was conducted within 30 days of MRI in the domains of psychomotor speed, attention/working memory, and executive function. A research staff member, trained and supervised by a board-certified neuropsychologist, administered the Grooved Pegboard Test (Dominant and Nondominant hands), Trail-Making Test (Parts A and B), Wechsler Adult Intelligence Scale–Revised (WAIS-R) Digit Span (Forward and Backward), and WAIS-R Digit Symbol Test. Test results were transformed to z-scores using appropriate age- and education-matched normative data.

Structural MRI Data Acquisition

Structural MRI data were acquired on a 3.0-T Philips Medical Systems Achieva machine equipped with an 8-channel head coil (InVision Imaging, Honolulu, HI). For each subject, a high-resolution anatomical volume was acquired with a sagittal T₁-weighted 3D turbo field echo (T1W 3D TFE) sequence (echo time [TE]/repetition time [TR] = 3.1/6.7 ms; flip angle 8°; slice thickness 1.2 mm with no gap; in-plane resolution 1.0 mm²; field of view 256 × 256 mm²; scan time = 10 min 13 s). Image files in DICOM format were transferred to a Linux workstation for morphometric analyses. MRS T₁-weighted and diffusion tensor imaging were included in the scanning protocol but not examined here.

Segmentation and Surface Extraction

The T₁-weighted structural MRI scans were processed using FreeSurfer v4.5.0 (Athinoula A. Martinos Center for Biomedical Imaging and CorpTechs Labs, http://www.nmr.mgh.harvard.edu/~freesurfer). Last accessed on 30, September 2011 as described at length in the literature (Dale and Sereno 1993; Dale et al. 1999; Fischl, Sereno, and Dale 1999; Fischl, Sereno, Tootell, et al. 1999; Fischl and Dale 2000; Fischl et al. 2001; Fischl et al. 2004; Segonne et al. 2004; Han et al. 2006; Jovicich et al. 2006). Briefly, the automated computationally intensive procedure includes skull-stripping intensity normalization, Talairach transformation, segmentation of subcortical white matter and deep
gray matter structures, and tessellation of the white matter surfaces. FreeSurfer uses a manually labeled prior segmentation to disambiguate subcortical structures. The gray/white surface is deformed outward, following intensity gradients and a constraint on curvature, to reconstruct the gray matter/cerebrospinal fluid boundary, or pial surface. The distance between the pial and the white matter surfaces yields an estimate of cortical thickness at each tessellation vertex. This method measures thickness of the cerebral cortex with great accuracy across the entire brain. The intersubject standard deviation of the thickness measure is less than 0.5 mm, enabling detection of focal atrophy in small populations or even individual subjects (Fischl and Dale 2000). The automated technique was validated histologically (Rasola et al. 2002) and by manual measurement on MRI sections (Kuperberg et al. 2003; Salat et al. 2004). Automated cortical parcellation was conducted within FreeSurfer using the Destrieux atlas (Destrieux et al. 2010).

**Group Analysis**

Prior to group analysis, the reconstructed cortical surfaces for each study participant were inspected for defects. FreeSurfer uses a measure of convexity to align the cortical surfaces and produce an average cortical surface. The measure of convexity is weighted such that folding pattern features such as the Sylvian fissure that show less variability across subjects have a higher weighting (Fischl, Sereno, Tootell, and Rosen 1998; Fischl, Sereno, Tootell, et al. 1999). A mapping was thus obtained between each vertex on the average surface and the corresponding vertex on the surface of each subject’s cortical reconstruction. The cortical thickness estimates for each subject were then resampled onto the average surface and smoothed with a 10-mm full-width/half-maximum Gaussian kernel. Subjects with undetectable and detectable HIV DNA levels constituted the 2 groups of interest. For each group, a linear regression model of cortical thickness as a function of age was computed at every vertex on the surface. The slopes of the lines were constrained to be the same for the 2 groups, but the vertical intercepts (representing thickness) were allowed to vary freely in obtaining the best fit to the data. Statistical significance of the between-group difference in vertical intercept, while regressing out the effect of age, was then inferred. The parametric maps directly display this P value measure of significance.

In order to extract mean cortical thickness values over the extended areas of thinning, we used FreeSurfer tools to delineate regions of interest (ROIs) on the average surface. The ROIs encompassed significant (P < 0.01) voxels on the parametric maps and included all the large regions where cortical thickness differences were identified by vertexwise analysis. Each ROI was then mapped to each individual subject’s surfaces, and mean cortical thickness over the ROI computed for each subject. Nonparametric statistical testing was subsequently performed on the mean cortical thickness values of the ROIs.

To summarize, the procedure was as follows: 1) at each point on the average surface, a linear regression (thickness against age) was performed for each group separately; 2) at each point on the average surface, we tested the significance of the cortical thickness intercept group differences to generate parametric maps; 3) we outlined ROIs on extended regions of significant statistical difference on the parametric maps, mapped the ROIs to each subject’s cortical surface, and for each subject, computed the mean cortical thickness over the ROI; and 4) for each ROI, we assessed the significance of group differences between these means using the nonparametric Mann-Whitney test. (Methods for multiple-comparison correction in linear models do not apply to nonparametric tests. However, if analysis of variance is used to evaluate significance of cortical thickness differences over the ROIs and a Bonferroni criterion is imposed [P < 0.0024 = 0.05/21], most group differences remain significant.)

**Statistical Methods**

Statistical analyses were conducted within Statview 5.0 (SAS Institute Inc., Cary, NC). We employed Mann-Whitney tests for HIV DNA group comparisons of subjects’ continuous demographic variables and neuropsychological z-scores. Dichotomous demographic characteristics were compared with chi-squared tests. Spearman rho correlations were used to assess relationships between regional brain thickness and variables characterizing demographics (e.g., age and education), disease severity (CD4 nadir count, years since HIV diagnosis, and current CD4 count), and neurobehavioral test performance (z-scores). Statistical significance was defined by P < 0.05 and trends by 0.05 ≤ P ≤ 0.1.

**Results**

**Subject Characteristics**

Nineteen subjects were included in these analyses (Table 1). Ten had undetectable levels of PBMC HIV DNA (<10 copies/10⁶ cells) and 9 had detectable HIV DNA (≥10 copies/10⁶ cells). The groups did not differ significantly in age, education, current or nadir CD4 cell count, or years since HIV diagnosis. All participants were on HAART. Plasma HIV RNA was undetectable (<50 copies/mL) in all but one patient; the exception was an individual in the detectable HIV DNA group whose plasma viral load was minimal at 158 copies/mL.

**Effect of HIV DNA Group Status on Cortical Thickness**

Parametric maps (Fig. 1) were calculated on the null hypothesis of no significant difference between the thickness intercepts (i.e., age = 0). The maps were generated using a lower threshold of P < 0.01 and a saturation point of P < 0.00001. With the exception of one tiny cluster in the left inferior parietal cortex (visible in blue in Fig. 1), all statistically significant voxel clusters showed thinner cortex in the group with detectable HIV DNA. Guided by these parametric maps, we defined ROIs that coincided with the regions of most significant cortical thickness change. Clusters with surface area <40 mm² were excluded from analysis. Locations of the ROIs are shown in Figure 2. Over each ROI, mean cortical thickness was computed for all subjects, and HIV DNA group differences were assessed using Mann-Whitney tests. Table 2 presents

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**Table 1**

Demographic and medical characteristics of HIV⁺ subjects with undetectable and detectable levels of PBMC HIV DNA

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Undetectable HIV DNA (&lt;10 copies/10⁶ cells)</th>
<th>Detectable HIV DNA (≥10 copies/10⁶ cells)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sample size</td>
<td>10</td>
<td>9</td>
<td>-</td>
</tr>
<tr>
<td>Sex (% male)</td>
<td>90</td>
<td>100</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Mean age (years)</td>
<td>54.6 ± 8.3</td>
<td>55.8 ± 5.5</td>
<td>0.81</td>
</tr>
<tr>
<td>Mean education (years)</td>
<td>14.5 ± 2.8</td>
<td>14.4 ± 2.4</td>
<td>0.97</td>
</tr>
<tr>
<td>Mean current CD4 count (cells/mm³)</td>
<td>549.7 ± 220.8</td>
<td>431.9 ± 198.4</td>
<td>0.19</td>
</tr>
<tr>
<td>Mean nadir CD4 count (cells/mm³)</td>
<td>183.4 ± 192.4</td>
<td>156.9 ± 146.1</td>
<td>&gt;0.99</td>
</tr>
<tr>
<td>Mean years since HIV⁺ diagnosis</td>
<td>145 ± 5.9</td>
<td>174 ± 8.9</td>
<td>0.31</td>
</tr>
<tr>
<td>Plasma HIV RNA (#undetectable)</td>
<td>10</td>
<td>8</td>
<td>0.47</td>
</tr>
<tr>
<td>Median PBMC HIV DNA (copies/10⁶ cells, min-max)</td>
<td>N/A</td>
<td>132 (29-28 901)</td>
<td>-</td>
</tr>
<tr>
<td>Race or ethnicity (Caucasian/non-Caucasian)</td>
<td>7/3</td>
<td>8/1</td>
<td>0.58</td>
</tr>
</tbody>
</table>

Note: P values are computed by Mann-Whitney (continuous variables) or chi-squared tests (categorical variables). N/A, not applicable.
cortical thickness values for the 21 ROIs. Differences between detectable and undetectable HIV DNA groups remained statistically significant ($P < 0.05$) in all ROIs when assessed nonparametrically. All ROIs demonstrated an association of detectable HIV DNA levels with cortical thinning. There was no significant Spearman correlation of cortical thickness with age, education, duration of illness, or current or nadir CD4. Although thickness of the cortex may not bear a linear relationship to actual HIV DNA burden, and our subjects with detectable HIV DNA ($N = 9$) were too few for a valid correlation statistic, we state for completeness that mean cortical thickness did not correlate with HIV DNA level for any ROI ($P > 0.1$).

The ROI analysis found the largest and most significant ($P < 0.001$) regions of cortical thinning to occur in the bilateral insula (left superior circular insular sulcus: ROI 1, -14%; right short insular gyrus: ROI 11, -16%). Small areas of right-hemisphere insular cortex (long and short gyrus and central sulcus: ROI 20) were approximately 10% significantly thinner in the detectable HIV DNA group. Decreased thickness of right-hemisphere cingulate cortex was notable in these subjects; the right anterior and midanterior cingulate (ROI 13) was 14% thinner ($P = 0.001$) when compared with the undetectable HIV DNA group.

Individuals with detectable HIV DNA demonstrated reduced thickness in frontal and parietal cortices relative to the comparison subjects. Most affected were the right superior frontal (ROI 14, $P = 0.001$) and left rostral middle frontal (ROI 4, $P = 0.004$) regions, which were 16% thinner. Significant cortical thinning was found in small areas of the right hemisphere (pars triangularis: ROI 18, -13%, $P = 0.009$). We also observed reduced thickness in orbitofrontal cortex. A large area of the right orbital gyrus and orbital medial olfactory sulcus (ROI 12) was about 16% thinner in detectable HIV DNA subjects ($P < 0.001$). Significant thinning was noted in the left orbital gyrus and H-shaped sulcus (ROI 5, -12%, $P = 0.003$).

In parietal cortex, the left supramarginal gyrus was significantly affected over both a large area (ROI 3, -11%, $P = 0.001$) and a smaller one (ROI 7, -15%, $P < 0.001$). Cortical thickness was reduced in the right precuneus (ROI 19, -15%, $P = 0.001$). Significant bilateral thinning was present in frontoparietal cortex: In the detectable HIV DNA group, paracentral gyral and sulcal thickness was decreased by approximately 13% (ROI 15, $P < 0.001$) in the right hemisphere and by 18% in the left (ROI 9, $P = 0.009$). Left precentral cortex was 11% thinner (ROI 8, $P < 0.001$).

Significant thinning associated with detectable HIV DNA occurred bilaterally in temporal lobes, particularly in the left hemisphere.
Subjects with detectable HIV DNA also showed significant thickness reductions in occipitotemporal cortex. Right-hemisphere fusiform and parahippocampal regions were affected (ROI 17, −10%, \( P < 0.001 \)), as were small areas of bilateral fusiform (right: ROI 21, −13%, \( P = 0.009 \); left: ROI 10, 19%, \( P = 0.018 \)).

It is worth noting that as derived by nonparametric statistical methods, the above-mentioned findings are in fact conservative. Parametric tests upheld the significant main effects of group; that is, \( t \)-tests yielded significant group differences in ROI cortical thickness, with \( P < 0.001 \) for most regions (Supplementary Table 1). Large effect sizes (typical Cohen’s \( d \sim 2.0 \)) probably enabled the detection of group differences with a small cohort. Most ROI group differences remained significant when a Bonferroni correction for multiple comparisons was applied (\( P < 0.0024 = 5/12 \)). The results (both nonparametric and parametric) were not skewed by the subject who had detectable HIV RNA, since excluding this individual from the analysis produced very little change in \( P \) values.

To investigate whether the apparent effects of HIV DNA may be linked to demographic or clinical factors, we added age, education, duration of illness, current CD4 count, and nadir CD4 count (separately) as independent variables in an analysis of covariance (ANCOVA). These parameters had nonsignificant effects on mean cortical thickness for virtually every ROI (\( P > 0.05 \)). Moreover, when the covariates were included in the model, effects of HIV DNA group status changed only minimally and did not change from significant to nonsignificant for any region. For example, we found no significant or trend-level effects of nadir or current CD4 count on mean cortical thickness (\( P > 0.1 \)) for the largest ROIs in each brain hemisphere (ROI 1−3, 11−15 of Supplementary Table 1). Age and education also had no significant effects (\( P > 0.05 \)). Age affected ROI 1 at trend-level (\( P = 0.0679 \)) but did not alter the effect of HIV DNA (\( P = 0.0001 \)). Similarly, while education showed trend effects on ROI 1 (\( P = 0.0701 \)) and ROI 14 (\( P = 0.0915 \)), adjusting for education resulted in HIV DNA group status becoming slightly more significant (\( P < 0.0001 \), ROI 1; \( P < 0.001 \), ROI 14).

### Table 2

<table>
<thead>
<tr>
<th>ROI</th>
<th>#Vertices</th>
<th>Hemisphere</th>
<th>Anatomical location</th>
<th>Mean cortical thickness (mm)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Undetectable HIV DNA</td>
<td>Detectable HIV DNA</td>
</tr>
<tr>
<td>1</td>
<td>496</td>
<td>L</td>
<td>Insula (superior circular sulcus)</td>
<td>2.76 ± 0.18</td>
<td>2.36 ± 0.16</td>
</tr>
<tr>
<td>2</td>
<td>224</td>
<td>L</td>
<td>Middle temporal gyrus</td>
<td>3.07 ± 0.34</td>
<td>2.49 ± 0.24</td>
</tr>
<tr>
<td>3</td>
<td>213</td>
<td>L</td>
<td>Superior temporal; inferior parietal (supramarginal gyrus)</td>
<td>2.97 ± 0.15</td>
<td>2.63 ± 0.17</td>
</tr>
<tr>
<td>4</td>
<td>132</td>
<td>L</td>
<td>Rostral middle frontal</td>
<td>2.12 ± 0.13</td>
<td>1.77 ± 0.25</td>
</tr>
<tr>
<td>5</td>
<td>91</td>
<td>L</td>
<td>Lateral orbitofrontal (orbital gyrus and H-shaped sulcus)</td>
<td>2.64 ± 0.17</td>
<td>2.31 ± 0.16</td>
</tr>
<tr>
<td>6</td>
<td>73</td>
<td>L</td>
<td>Superior temporal sulcus</td>
<td>2.62 ± 0.31</td>
<td>2.25 ± 0.21</td>
</tr>
<tr>
<td>7</td>
<td>67</td>
<td>L</td>
<td>Supramarginal gyrus</td>
<td>2.87 ± 0.19</td>
<td>2.44 ± 0.20</td>
</tr>
<tr>
<td>8</td>
<td>49</td>
<td>L</td>
<td>Precentral (central sulcus)</td>
<td>2.12 ± 0.12</td>
<td>1.98 ± 0.08</td>
</tr>
<tr>
<td>9</td>
<td>43</td>
<td>L</td>
<td>Paracentral gyrus and sulcus</td>
<td>2.40 ± 0.30</td>
<td>1.97 ± 0.22</td>
</tr>
<tr>
<td>10</td>
<td>40</td>
<td>L</td>
<td>Fusiform</td>
<td>2.24 ± 0.35</td>
<td>1.82 ± 0.11</td>
</tr>
<tr>
<td>11</td>
<td>321</td>
<td>R</td>
<td>Insula (short gyrus)</td>
<td>3.83 ± 0.21</td>
<td>3.20 ± 0.31</td>
</tr>
<tr>
<td>12</td>
<td>313</td>
<td>R</td>
<td>Orbital gyrus; medial orbital olfactory sulcus</td>
<td>2.56 ± 0.15</td>
<td>2.16 ± 0.14</td>
</tr>
<tr>
<td>13</td>
<td>311</td>
<td>R</td>
<td>Anterior and midanterior cingulate gyrus and sulcus</td>
<td>2.95 ± 0.18</td>
<td>2.53 ± 0.23</td>
</tr>
<tr>
<td>14</td>
<td>301</td>
<td>R</td>
<td>Superior frontal gyrus</td>
<td>3.00 ± 0.27</td>
<td>2.53 ± 0.16</td>
</tr>
<tr>
<td>15</td>
<td>124</td>
<td>R</td>
<td>Precentral gyrus and sulcus</td>
<td>2.10 ± 0.12</td>
<td>1.90 ± 0.13</td>
</tr>
<tr>
<td>16</td>
<td>107</td>
<td>R</td>
<td>Temporal pole</td>
<td>4.09 ± 0.29</td>
<td>3.69 ± 0.22</td>
</tr>
<tr>
<td>17</td>
<td>105</td>
<td>R</td>
<td>Fusiform; parahippocampal</td>
<td>2.46 ± 0.09</td>
<td>2.23 ± 0.10</td>
</tr>
<tr>
<td>18</td>
<td>70</td>
<td>R</td>
<td>Pars triangularis</td>
<td>2.61 ± 0.22</td>
<td>2.27 ± 0.18</td>
</tr>
<tr>
<td>19</td>
<td>66</td>
<td>R</td>
<td>Precuneus</td>
<td>2.51 ± 0.26</td>
<td>2.13 ± 0.19</td>
</tr>
<tr>
<td>20</td>
<td>52</td>
<td>R</td>
<td>Insula (central insular sulcus; long and short insular gyri)</td>
<td>3.23 ± 0.20</td>
<td>2.91 ± 0.11</td>
</tr>
<tr>
<td>21</td>
<td>40</td>
<td>R</td>
<td>Fusiform</td>
<td>3.38 ± 0.28</td>
<td>2.92 ± 0.32</td>
</tr>
</tbody>
</table>

Note: L, left; R, right.
Only duration of illness had a significant effect on mean cortical thickness in any region ($P = 0.0472$, ROI 14), as inclusion of this variable in the ANCOVA increased the significance of HIV DNA ($P < 0.0001$, ROI 14). Therefore, the significant main effects of HIV DNA category persisted even when disease history, current disease status, or demographics were covaried as potentially confounding variables, in agreement with the lack of Spearman correlation between these parameters and ROI cortical thickness.

### Cortical Thinning and Behavioral Effects

Using the Mann–Whitney Test, we found a significant HIV DNA group effect on $z$-scores for the Grooved Pegboard Test with Dominant Hand (mean = 0.45, undetectable HIV DNA; mean = –0.51, detectable HIV DNA; $P = 0.014$). Detectable and undetectable HIV DNA groups did not perform significantly differently on Digit Symbol, Grooved Pegboard (Nondominant Hand), or Trail-Making A or B Tests.

The relation between mean ROI cortical thickness values and Grooved Pegboard (Dominant) Test $z$-scores was explored using Spearman rho correlations (Table 3). Thinner cortex correlated significantly with poorer test performance (i.e., lower $z$-scores or higher peg insertion times) in most of the ROIs, notably the left insula (ROI 1, $P = 0.007$, $\rho = 0.64$). We also found moderately strong correlations between lower $z$-scores and decreased cortical thickness of other temporal regions (left supramarginal gyrus: ROI 7, $P = 0.007$, $\rho = 0.63$; left fusiform: ROI 10, $P = 0.015$, $\rho = 0.57$; right cingulate gyrus and sulcus: ROI 13, $P = 0.015$, $\rho = 0.58$; and right temporal pole: ROI 16, $P = 0.019$, $\rho = 0.55$) in parietal cortex, thinning of the right paracentral gyrus and sulcus (ROI 15, $P = 0.003$, $\rho = 0.71$) and the right precuneus (ROI 19, $P = 0.009$, $\rho = 0.62$) correlated with diminished test performance.

### Discussion

We demonstrated a significant association of regional cortical thinning with detectable levels of PBMC HIV DNA in virally suppressed HIV$^+$ individuals. The comparison group comprised age- and education-matched HIV$^+$ subjects with undetectable HIV DNA; we emphasize that they were not healthy controls. Global thinning of the cortical ribbon was visually apparent in all study participants. Thus, the spatial pattern of gray matter loss described here is superposed on cortical alterations present in individuals whose viral DNA is undetectable. Prefrontal and frontoparietal cortices were affected in subjects with elevated HIV DNA, consistent with prior studies of HIV-related brain atrophy, and we also detected thinning of temporal gray matter. Of particular interest is the insula, a structure not previously considered a target of this disease.

Worldwide, both with and without HAART (Robertson et al. 2009), impairment of executive function, motor skills, attention, working memory, and speed of information processing tends to be pronounced in HIV infection (Stout et al. 1995; York et al. 2001; Marcotte et al. 2006; Dawes et al. 2008). The specific pattern of cognitive deficits varies greatly across individuals (Dawes et al. 2008). Under HAART, diminished cognitive ability may not correlate with plasma HIV RNA levels (Robertson et al. 1998; Lawrence and Major 2002; Albright et al. 2003; Langford et al. 2003; McArthur et al. 2003; Rege et al. 2005) but is linked to elevated HIV DNA (Shiramizu et al. 2005, 2007a, 2007b, 2009), which can persist in reservoirs despite treatment (Dickover et al. 1992; Bruisten et al. 1998; Delobel et al. 2005). Comparing HIV$^+$ subjects who had normal cognition, minor cognitive motor disorder, and HIV-associated dementia, Shiramizu et al. (2009) found that the amount of PBMC HIV DNA at study entry was proportional to HIV-associated neurocognitive impairment in all 3 groups. Moreover, viral DNA level was associated with deficits in individual cognitive domains that included motor speed and working memory. Recognition memory suffered the greatest decline.

In the present cross-sectional study, which employed limited neuropsychological testing, only Grooved Pegboard (Dominant Hand) Test scores differed significantly between subjects with undetectable and detectable HIV DNA. Impaired psychomotor speed as assessed by this task correlated strongly with cortical thinning in multiple affected regions involved in motor speed, such as the supramarginal gyrus and anterior cingulate cortex (Naito et al. 2000), or in motor control; for example, the precuneus (Jancke et al. 2000; Witt et al. 2008). A larger study may reveal relationships between cortical alterations and other measures of cognitive function. Therefore, we discuss below the relevance of brain areas identified in this paper to neurocognitive and motor impairment in the HIV population. Our focus is on regional cortical functions pertaining to the cognitive domains (recognition memory, visuospatial ability, learning, verbal memory, executive function, working memory, attention and concentration, visual memory, and language) in which deficits were correlated with PBMC HIV DNA levels (Shiramizu et al. 2009).

Our study found the largest areas of cortical thinning to be located in the bilateral anterior insula. The insula is considered a supplementary motor (Augustine 1985) or motor association area (Augustine 1996; Borovsky et al. 2007). Left and right anterior insula are linked to vocal motor control of speech production (Ackermann and Riecker 2003; Borovsky et al. 2007), respectively, and bilateral insular injury can dramatically disrupt both verbal and nonverbal communication (Habib et al. 1995). The role of this structure, however, is intriguingly complex. Among a vast range of functions, the insula is

<table>
<thead>
<tr>
<th>ROI</th>
<th>Hemisphere</th>
<th>Anatomical region</th>
<th>$P$</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L</td>
<td>Insula (superior circular sulcus)</td>
<td>0.64</td>
<td>0.007</td>
</tr>
<tr>
<td>2</td>
<td>L</td>
<td>Middle temporal gyrus</td>
<td>0.31</td>
<td>0.193</td>
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<td>L</td>
<td>Superior temporal, inferior parietal (supramarginal gyrus)</td>
<td>0.86</td>
<td>0.386</td>
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<tr>
<td>4</td>
<td>L</td>
<td>Rostral middle frontal</td>
<td>0.39</td>
<td>0.096</td>
</tr>
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<td>5</td>
<td>L</td>
<td>Lateral orbitofrontal (orbital gyrus and H-shaped sulcus)</td>
<td>0.49</td>
<td>0.037</td>
</tr>
<tr>
<td>6</td>
<td>L</td>
<td>Superior temporal sulcus</td>
<td>0.44</td>
<td>0.063</td>
</tr>
<tr>
<td>7</td>
<td>L</td>
<td>Supramarginal gyrus</td>
<td>0.63</td>
<td>0.008</td>
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<tr>
<td>8</td>
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<td>Precuneal (central sulcus)</td>
<td>0.55</td>
<td>0.021</td>
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<tr>
<td>9</td>
<td>L</td>
<td>Paracentral gyrus and sulcus</td>
<td>0.40</td>
<td>0.088</td>
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<tr>
<td>10</td>
<td>L</td>
<td>Fusiform</td>
<td>0.57</td>
<td>0.015</td>
</tr>
<tr>
<td>11</td>
<td>R</td>
<td>Insula (short gyrus)</td>
<td>0.36</td>
<td>0.130</td>
</tr>
<tr>
<td>12</td>
<td>R</td>
<td>Orbital gyrus; medial orbital alfactory sulcus</td>
<td>0.43</td>
<td>0.066</td>
</tr>
<tr>
<td>13</td>
<td>R</td>
<td>Anterior and midanterior cingulate gyrus and sulcus</td>
<td>0.58</td>
<td>0.015</td>
</tr>
<tr>
<td>14</td>
<td>R</td>
<td>Superior frontal gyrus</td>
<td>0.36</td>
<td>0.130</td>
</tr>
<tr>
<td>15</td>
<td>R</td>
<td>Paracentral gyrus and sulcus</td>
<td>0.71</td>
<td>0.003</td>
</tr>
<tr>
<td>16</td>
<td>R</td>
<td>Temporal pole</td>
<td>0.55</td>
<td>0.019</td>
</tr>
<tr>
<td>17</td>
<td>R</td>
<td>Fusiform; parahippocampal</td>
<td>0.44</td>
<td>0.061</td>
</tr>
<tr>
<td>18</td>
<td>R</td>
<td>Pars triangularis</td>
<td>0.40</td>
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<td>19</td>
<td>R</td>
<td>Precuneus</td>
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<td>0.009</td>
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<td>Insula (central insular sulcus; long and short insular gyri)</td>
<td>0.47</td>
<td>0.045</td>
</tr>
<tr>
<td>21</td>
<td>R</td>
<td>Fusiform</td>
<td>0.26</td>
<td>0.282</td>
</tr>
</tbody>
</table>

Note: Regions in bold correlate significantly ($P < 0.05$) with Grooved Pegboard (Dominant Hand) Test $z$-scores. L, left; R, right.
implicated in somatosensory perception (Fink et al. 1997), emotional processing (Phan et al. 2002), and self-awareness (Tsakiris et al. 2007; Craig 2009). As a nodal point between limbic and motor systems (Ackermann and Riecker 2004), the insula is believed to integrate autonomic, affective, sensory, and cognitive input to create representations of affective state (Jones et al. 2010).

Thinner insular cortex in our cohort correlated with poorer performance on the Grooved Pegboard Test for Dominant Hand. This finding is consistent with the insula’s apparent involvement in paced tasks and oculomotor control (Anderson et al. 1994) and hand motor control (Fink et al. 1997). More generally, with the right anterior insula implicated as a central node in both dorsal and ventral attention systems (Eckert et al. 2009), an association between elevated HIV DNA and insular atrophy may manifest itself in various types of neuropsychological deficits (e.g., attention, concentration, and working memory) among subjects with detectable viral DNA.

Executive function performance is one of the cognitive domains significantly correlated with higher HIV DNA levels (Shiramizu et al. 2009). Another is recognition memory. The known effects of insular lesions include profound deficits in risk assessment (Jones et al. 2010) that have clear implications for executive functioning. Data from lesions appear to confirm the role of the insula in executive function (Clark et al. 2008), supporting the somatic marker hypothesis that decision making relies on processing and integration of emotionally relevant information (Damasio 1994; Bechara and Damasio 2002). Functional MRI (fMRI) studies of healthy volunteers have linked activation of the anterior insula to decision making under uncertainty or risk (Paulus et al. 2003; Kuhnen and Knutson 2005). Insular atrophy could thus result in executive dysfunction. Damaged insula in individuals with elevated HIV DNA may also contribute to impaired recognition memory: in a striking cause-and-effect experiment, administration of the muscarinic cholinergic receptor antagonist scopolamine into the insular cortex of rats produced a deficit in visual object recognition memory (Bermudez-Rattoni et al. 2005).

The insula is connected to cingulate cortex, orbitofrontal cortex, the temporal pole, and superior temporal sulcus (Augustine 1985, 1996; Ongur and Price 2000). In all of these regions, we found significantly reduced cortical thickness in subjects with detectable HIV DNA, atrophy which may have a bearing on specific neuropsychological deficits. The right caudal anterior cingulate, for example, has been implicated in error detection (Pourtois et al. 2010; Simoes-Franklin et al. 2010). Impairment of error monitoring, an executive function, would influence other neurocognitive domains affected by elevated HIV DNA, such as recognition memory, motor skills and motor speed. Orbitofrontal cortex also contributes to decision making (Wallis 2007). Damage to medial orbitofrontal and anterior cingulate cortex is known to affect judgment and impulse control (Berlin et al. 2004; Cato et al. 2004).

Higher HIV DNA levels correlate with diminished verbal and visual memory (Shiramizu et al. 2009), functions mediated by the temporal lobe (Libon et al. 2009; Maki et al. 2009). We found detectable HIV DNA to be associated with bilateral thinning of temporal cortex. Cortical thinning of brain regions identified in our study, including the insula, is exhibited by patients with semantic dementia and progressive nonfluent aphasia (Rohrer et al. 2009).

Another site of decreased cortical thickness in our detectable HIV DNA group is the precuneus, in the posterior medial parietal lobe. The precuneus may participate in brain networks underlying recognition memory (Dorfel et al. 2009). Furthermore, HIV is associated with significant impairment of parietal-dependent visuospatial skills (Olesen et al. 2007) and damage to the right-hemisphere precuneus linked to loss of navigational ability (Suzuki et al. 1998). Data from positron emission tomography (PET) indicate that navigation may be subverted by a network that includes the insula and precuneus (Ghaem et al. 1997). PET and MRI results are converging on a central role of the precuneus in motor and visuospatial imagery (Cavanna and Trimble 2006). Since HIV+ subjects have shown altered frontoparietal network activation (Ernst et al. 2002; Chang, Tomasi, et al. 2004; Castelo et al. 2006), the pattern of cortical thinning that we have found may reflect disrupted brain circuitry.

In reviewing the cognitive functions of brain regions implicated in our work, we have emphasized the largest area, the insula, and touched on only a small fraction of relevant research. There is a vast literature on substantiated and posited functions of all the affected regions. It is probable that damage to these cortical areas underlies the increased burden of neurocognitive deficits seen in individuals with detectable HIV DNA. Two caveats, however, apply. This cross-sectional study does not permit inference of a causal relationship between elevated HIV DNA and cortical thinning. Moreover, although nonparametric and parametric procedures gave similar results, our small sample size limited the applicability of parametric statistical analysis. A large, carefully designed study, with the power to include main and interaction effects of all relevant clinical parameters in a multivariate model, is needed to confirm associations among cortical thickness, HIV DNA, and cognitive function.

Conclusions

Using a direct validated measurement of cortical thickness, we have identified a statistically significant pattern of cortical thinning in subjects with detectable levels of HIV DNA. Given the prevalence of neurocognitive sequelae present in populations of HAART-treated HIV+ individuals, the importance of uncovering a neural basis for these deficits is paramount in the search for effective therapies. The statistical significance of the cortical thinning pattern, as well as the fact that we found a neuropsychological testing correlate, lends credence to the results. Much of the affected cortex is located in the frontal lobe where previous HIV/AIDS research identified the most prevalent damage. In this study, we did not examine subcortical regions or white matter, but alterations in these brain structures are also probable. The pattern of cortical thinning presented here, likely related to cognitive deficits associated with elevated HIV DNA, may indicate disrupted attentional networks in HIV although the findings extend across regions that possibly comprise a number of different functional systems. Open questions remain as to whether the changes in cortical thickness reflect neuronal death or loss of neuropil, and whether they are due to HIV-related toxins or perhaps to a diaschisis effect (possibly mediated by white matter destruction) resulting from compromised subcortical brain structures.

HIV DNA may prove a useful marker of HIV-related brain injury and treatment efficacy, particularly in patients whose
plasma HIV RNA levels fall below the detectable threshold. Our findings support initiation of HAART during the primary phase of infection for optimal depletion of the PBMC HIV DNA reservoir. Obvious limitations of this work are the small sample size and limited neuropsychological tests. Future longitudinal studies are needed to confirm our results and should be conducted using a large study population and extensive neuropsychological testing. High angular resolution diffusion imaging and resting-state fMRI acquisitions may be useful modalities for investigating white matter damage and the possible association of detectable HIV DNA levels with altered brain structural and functional connectivity. Further research should include subset analyses to localize HIV DNA to monocytes and lymphocytes.

Supplementary Material
Supplementary material can be found at: http://www.cercor.oxfordjournals.org/

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